Analysis of genetic and phenotypic heterogeneity in juvenile polyposis


Abstract

Background—Juvenile polyposis syndrome (JPS) is characterised by gastrointestinal (GI) hamartomatous polyposis and an increased risk of GI malignancy. Juvenile polyps also occur in the Cowden (CS), Bannayan-Ruvalcaba-Riley (BRRS) and Gorlin (GS) syndromes. Diagnosing JPS can be problematic because it relies on exclusion of CS, BRRS, and GS. Germline mutations in the PTCH, PTEN and DPC4 (SMAD4) genes can cause CS, GS/BRRS, and JPS, respectively.

Aims—To examine the contribution of mutations in PTCH, PTEN, and DPC4 (SMAD4) to JPS.

Methods—Forty seven individuals from 15 families and nine apparently sporadic cases with JPS were screened for germline mutations in DPC4, PTEN, and PTCH.

Results—No patient had a mutation in PTEN or PTCH. Five different germline mutations were detected in DPC4; three of these were deletions, one a single base substitution creating a stop codon, and one a missense change. None of these patients had distinguishing clinical features.

Conclusions—Mutations in PTEN and PTCH are unlikely to cause juvenile polyposis in the absence of clinical features indicative of CS, BRRS, or GS. A proportion of JPS patients harbour DPC4 mutations (21% in this study) but there remains uncharacterised genetic heterogeneity in JPS.

Keywords: juvenile polyposis syndrome; germline mutations

Juvenile polyposis (JPS; MIM 174 900) is a rare autosomal dominant condition characterised by hamartomatous polyps, usually within the colon but occasionally arising in the stomach and small bowel.1 These polyps are typified by a predominant stroma, cystic spaces, and an abundant lamina propria lacking smooth muscle, so distinguishing them from Peutz-Jeghers polyps. Unlike solitary juvenile polyps which may affect up to 2% of children and adolescents and have little or no malignant potential, JPS patients have an increased risk of gastrointestinal malignancy.2 3 JPS may occur in association with arteriovenous malformations (MIM 175 050) but it is not clear if this represents a distinct syndrome.

Juvenile polyps also occur as a manifestation of the dominantly transmitted familial cancer syndromes: Cowden syndrome (CS; MIM 158 350) characterised by multiple hamartomas, macrocephaly, trichilemmomas, and a high risk of benign and malignant neoplasms of the thyroid, breast, uterus and skin; Bannayan-Ruvalcaba-Riley syndrome (BRRS), BZS; MIM 153 480 characterised by mental retardation, macrocephaly, lipomatosis, haemangiomas and genital pigmentation; and Gorlin syndrome (GS; MIM 109 400) characterised by multiple naevus basal carcinomas, skeletal abnormalities, and odontogenic keratinocites, macrocephaly, intracranial calcification, and craniofacial abnormalities. Compared with JPS the risk of gastrointestinal malignancy in CS, BRRS, and GS appears to be low.4

GS results from germline mutations in the PTCH gene (homologue of Drosophila patched) on chromosome 9q22.1.5 Juvenile polyps appear to comprise a relatively minor and infrequent component of this disease although few GS patients undergo gastrointestinal screening. Nevertheless, PTCH remains a good candidate for JPS given the possibility that a different spectrum of mutations might cause juvenile polyps without the other features of GS. No study to date has tested PTCH for germline mutations in JPS patients.

The CS gene is PTEN (phosphatase and tensin homologue deleted on chromosome 10 (10q22-q23)), a ubiquitously expressed dual specificity phosphatase that acts as a tumour suppressor and is mutated in several sporadic tumour types.6 7 The inference from patients’ clinical features that CS and BRRS might represent allelic forms of the same disease is supported by demonstration of germline mutations in PTEN in some but not all patients with BRRS.8 The shared clinical features of CS/BRRS and JPS, coupled with coincident somatic mutation data in juvenile polyps,8 raised the possibility that PTEN could cause all of these syndromes. This hypothesis has been

Abbreviations used in this paper: JPS, juvenile polyposis syndrome; CS, Cowden syndrome; BRRS, Bannayan-Ruvalcaba-Riley syndrome; GS, Gorlin syndrome; CSGE, conformation specific gel electrophoresis; SSCP, single strand conformational gel polymorphism.
Genetic and phenotypic heterogeneity in juvenile polyposis

Molecular Genetics Laboratory, Pathology Department, Dunedin School of Medicine, Dunedin, New Zealand D Markie
Roswell Park Cancer Institute, Buffalo, New York 14265, USA M A Rodríguez-Bigas
Department of Clinical Genetics, Royal Hospital for Children, St Michael's Hill, Bristol, UK E Sheridan
Centre for Polyposis and Inflammatory Bowel Diseases, 1–5–45, Yushina, Bunkyo-Ku, Tokyo 113, Japan T Iwama
Wessex Regional Genetics Service, Southampton SO16 5YA, UK D Eccles
Department of Histopathology, Worcester Royal Infirmary, WR1 3AS, UK G T Smith
Department of Paediatrics, University of Ulster College of Medicine, and Asan Institute, Seoul, Korea J C Kim
Institute of Medical Genetics, University of Wales College of Medicine, Cardiff CF4 4XN, UK J R Sampson
Regional Genetic Service, St Mary's Hospital, Manchester M13 0JH, UK G Evans
Centre for Human Genetics, Campus Gasthuisberg, O and N6, B-3000, Leuven, Belgium S Teijpar
Cancer Immuno- genetics Laboratory, ICRF, Institute of Molecular Medicine, Oxford OX3 9DS, UK W F Bodmer

Correspondence to: R Houlston, Haddow Laboratories, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK. Email: r.houlston@icr.ac.uk
Accepted for publication 25 November 1999

pursued by a number of workers with differing findings.24–22 Some studies found germ line
PTEN mutations in individuals who had been diagnosed as having JPS but there is doubt that a
diagnosis of CS/BRRS had been formally excluded in these patients. We have previously
found no PTEN mutations in a relatively large set of JPS patients (including a subset of the
cases reported below).

Recently it has been shown that constitutional
mutations in DPC4 (deleted in pancreatic
cahmatic locus 4; also referred to as SMAD4, small mothers against decapentaple-
gic deleted in pancreatic carcinoma, locus 4) can cause JPS.23-24 This gene, which codes for a
protein involved in transforming growth factor β1 signal transduction, is mutated in a number
of gastrointestinal carcinomas.25–28 Previous studies have reported DPC4 mutations in 5/9 (56%) and 1/21 (5%) JPS patients (the latter study including a subset of the cases reported below).

Other members of the SMAD family are
candidates for JPS but no mutations in
SMAD1/2/3/5/6/7 have been found in JPS patients.29

Hereditary mixed polyposis syndrome
(HMPS, MIM 601 228) is also characterised by atypical juvenile polyps with mixed features of
hamartomatous and adenomatous.30 No gene for
this syndrome has been identified but linkage
to chromosome 6q has been reported in one
large family.31

Hence there is evidence of considerable
genetic heterogeneity in JPS. We have studied
56 individuals with a presumptive diagnosis of
JPS, comprising 47 cases from 15 JPS families and nine apparently sporadic cases. These
patients comprise an exceptionally large set of
JPS cases. Our aims were: (i) to describe the
clinical features of JPS patients (including features overlapping with CS, BRRS, and GS); (ii) to resolve the debate as to whether or not
PTEN mutations account for any cases of JPS;
(iii) to test PTCH as a candidate gene for JPS;
(iv) to determine the contribution of DPC4
mutations to JPS; (v) to assess the existence of
any associations between germine mutations
and clinical features; and (vi) to determine the
proportion of JPS cases caused by as yet
unidentified genes.

Patients and methods

Patients with JPS were identified from sources in
the UK, Australia, Israel, USA, Japan, and Korea. The diagnostic criteria for JPS used in
this study were in accordance with the proposal of
Ko and colleagues32 that affected individuals
have either five or more juvenile polyps
throughout the gastrointestinal tract or any
number of juvenile polyps and a family history
of JPS. All affected individuals had more than
one typical juvenile polyp as confirmed by his-
tology. The following clinical data were also
obtained using a standardised proforma: family
history; occurrence of benign and malignant
tumours; dysmorphic features; arteriovenous
malformations and other cardiovascular
anomalies; pigmentation; and other notable
clinical features including those of CS, BRRS,
and GS. Where possible, material from JPS
polyps and other tumours was obtained for
histological review and confirmation of diagno-

Constitutive DNA from individuals was
extracted from EDTA blood samples using a
standard sucrose lysis method. Published
oligonucleotide and reaction conditions were
used to amplify specifically each exon of the
dPCR, PTEN, and PTCH genes.20 24 31 The
search for germline mutations in DPC4 was
performed using conformation specific gel
electrophoresis (CSGE). Two independent
workers using either CSGE or single strand
conformational gel polymorphism (SSCP)
undertook screening for mutations in PTEN. A
combination of CSGE and SSCP was used to
screen the PTCH gene. All samples with band
shifts were sequenced in duplicate and in
forward and reverse orientations, after replami-
ification of the appropriate exon from genomic
dNA in the polymerase chain reaction, using
the ABI Ready Reaction Dye Terminator Cycle
Sequencing kit and the 377 Prism sequencer.

Results

Fifty six patients with JPS were studied; 47
were ascertained from 15 families and nine
apparently sporadic patients had no known
relatives affected with JPS, although in three
cases there was a strong family history of colo-
rectal cancer. Table 1 details the clinical
characteristics of all patients. Mean age at
presentation of juvenile polyposis was 23 (SD
16) years (range 1–65). All affected individuals
from whom histological material was available
had juvenile polyps of a typical type.

The extent of polyposis within the gastro-
intestinal tract varied considerably between
groups in terms of both the numbers and sites
of polyp formation. Some of the families
showed marked evidence of intrafamilial differ-
ences in disease expression (table 1) even
allowing for factors such as patient age and
screening by colonoscopy. Furthermore, in a
number of patients there was documented evi-
dence of adenomatous intestinal polyposis
although none had the atypical juvenile polyps
found in HMPS.

The increased risk of gastrointestinal cancer
reported in association with JPS was clearly
present in our patients. Fifteen (27%) had
developed some type of gastrointestinal cancer
by the age of 65. Of these, six were small bowel
carcinomas, two gastric cancers, and seven
colorectal cancers. Based on cancer incidence
rates in the UK,32 this equates to about a
16-fold increased risk of gastrointestinal malign-
ancy in JPS.

None of the patients had prototypical
dermatological (e.g. trichoepitheliomas) or skel-
etal phenotypic features indicative of CS,
BRRS, or GS. There was some overlap
between the clinical features of JPS patients
and these other syndromes—three patients had
macrocephaly for example—but no specific
features to suggest misdiagnosis. Furthermore,
none of the patients had a past history of thyroid
cancer and only one had developed

breast cancer. Three patients had hyper- or
hypothyroidism but no tissue diagnosis suggestive of CS. The increased risk of cancer in these JPS patients appeared to be confined to the gastrointestinal tract in contradistinction to CS and GS. Arteriovenous and other cardiovascular anomalies were present in three patients (5%). The reported association between JPS and these anomalies may therefore be genuine.

### Table 1  Clinical features of the patients studied

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Age*</th>
<th>Sex</th>
<th>Gastrointestinal tract**</th>
<th>Skin</th>
<th>Skeletal</th>
<th>CNS</th>
<th>Cardiac</th>
<th>Breast</th>
<th>Thyroid</th>
<th>Other</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>W 3</td>
<td>47 F</td>
<td>Caecal Ca aged 47, jejunal JPs and adenomas, TA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W 2</td>
<td>14 F</td>
<td>100+JPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W 1</td>
<td>32 F</td>
<td>Ca colon (transverse) aged 32, (mixed, Ad and JPs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK 1</td>
<td>13 F</td>
<td>Multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRO 1</td>
<td>13 F</td>
<td>Multiple JPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 1</td>
<td>29 F</td>
<td>(50+) JPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV 1</td>
<td>12 M</td>
<td>Sigmoid and rectal polyps (50+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SH 1</td>
<td>1 M</td>
<td>Multiple colonic polyps, resection aged 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC 1</td>
<td>16 M</td>
<td>Colonic (30-50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD 1</td>
<td>15 M</td>
<td>Jejunal polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR 1</td>
<td>4 F</td>
<td>Recto-sigmoid and Caecal polyps (50+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF 1</td>
<td>16 M</td>
<td>Colonic polyps (4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCA 1</td>
<td>27 F</td>
<td>Multiple sigmoid polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 3.5</td>
<td>37 M</td>
<td>Colonic and gastric polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2 6</td>
<td>M</td>
<td>Beal, jejunal, colonic, and rectal polyps; Ca ileum; Ca stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3 65 F</td>
<td>F</td>
<td>Colonic and rectal polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 102 F</td>
<td>41 F</td>
<td>Ca colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>202 21 M</td>
<td>Ileum and colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201 24 M</td>
<td>Multiple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 204 32 2</td>
<td>F</td>
<td>Small bowel, colonic and gastric polyps (JPs and Ad, 20+); Ca ileum aged 49; intussusception</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>302 16 M</td>
<td>Multiple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>301 21 F</td>
<td>Colonic polyps (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 302 23 6</td>
<td>M</td>
<td>Colonic and small intestinal polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>402 9 F</td>
<td>Colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201 55 F</td>
<td>Colonic polyps (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>401 12 M</td>
<td>Colonic polyps (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 4.4 18 M</td>
<td>Colonic polyps (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5 30 M</td>
<td>Colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 1.2 50 F</td>
<td>Ca colon aged 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 20 M</td>
<td>Caecal polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 18 F</td>
<td>Multiple JPs; Ca stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 301 17 M</td>
<td>Colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203 19 F</td>
<td>JPs and Ad polyps in colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>302 4 M</td>
<td>Colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 502 14 5</td>
<td>F</td>
<td>Multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>305 51 F</td>
<td>Ca colon (transverse) aged 51, multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>306 43 M</td>
<td>Colonic polyps (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>308 61 M</td>
<td>Caecal Ca aged 61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>405 48 F</td>
<td>Multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 204 8 M</td>
<td>Multiple colonic polyps; Ca jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>206 8 M</td>
<td>Multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201 7 M</td>
<td>Polyposis at autopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>307 4 F</td>
<td>Florid colonic polyposis; small intestinal polyps (22); Ca small intestine aged 27.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>304 22 7 F</td>
<td>Caecal and colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>308 17 M</td>
<td>Multiple (TA, Ad and JP) in colon and duodenum; Ca jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>303 7 M</td>
<td>Ca jejunum aged 37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 102 F</td>
<td>Ca colon aged 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>301 13 F</td>
<td>20+ polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>302 14 M</td>
<td>Multiple colonic polyps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201 32 M</td>
<td>Multiple polyps in colon and stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 202 NK M</td>
<td>&gt;100 gastric JPs, 8 colorectal JPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203 NK M</td>
<td>Gastric JPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 201 NK F</td>
<td>Extensive polyposis, colectomy aged 45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>301 NK F</td>
<td>Colorectal JPs; colectomy aged 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL 101 39 M</td>
<td>Colonic polyps (TA + Ad)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201 6 F</td>
<td>&gt;100 JP; exocrine pancreatic insufficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*At diagnosis (NK, not known); **Number of polyps in parentheses; ***Familial case but only index case analysed.

Ad, adenoma; AR, aortic regurgitation; AS, aortic stenosis; BBD, benign breast disease; BK, bifid kidney; Ca, Cancer; CD, conduction defect; CLP, cleft palate; CL, clinodactyly; CO, coarctation; FK, excessive freckling; HT, hypertelorism; Hyper, hyperthyroidism; Hypo, hypothyroidism; JPs, juvenile polyps; MC, marcocephaly; ML, multiple lipomas; OVC, ovarian cyst; P, poryphria; PD, polydactyly; SAH, subarachnoid haemorrhage; Schiz, schizophrenia; SR, Schatski ring; TA, tubular adenoma; VSD, ventricular septal defect.
Table 2 Description of DPC4 mutations identified in JPS patients

<table>
<thead>
<tr>
<th>Patient/ family</th>
<th>Mutation</th>
<th>Predicted effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV</td>
<td>11bp deletion (GTCCACTGAA) at nucleotide 516 (exon 4)</td>
<td>Frameshift creating stop codon at nucleotide 561</td>
</tr>
<tr>
<td>AF</td>
<td>CGC to TGC, at nucleotide 1083 (exon 8)</td>
<td>Arg to Cys</td>
</tr>
<tr>
<td>17</td>
<td>2 bp deletion (CC) at nucleotide 1564 (exon 11)</td>
<td>Frameshift creating stop codon at nucleotide 1575</td>
</tr>
<tr>
<td>20</td>
<td>9 bp deletion (AAATTGAGG) at nucleotide 189 (exon 1)</td>
<td>Deletion of amino acids 64-66</td>
</tr>
<tr>
<td>BL</td>
<td>CGA to TGA at nucleotide 1333 (exon 10)</td>
<td>Substitution creating stop codon</td>
</tr>
</tbody>
</table>

No JPS patient had a germline PTEN mutation. Control samples with known PTEN mutations showed aberrant SSCP bands and previously reported polymorphisms were detected. PTEN mutations are typically found in 80% of CS and 50% of BRRS cases which adhere to operational diagnostic criteria established by the International Cowden Consortium.31 Also, germline PTCH mutations were not detected in our JPS cases. PTCH mutations are typically detectable in 39% of GS patients.31

Five germline DPC4 mutations were identified (table 2). Three of these were deletions ranging in size from two to 11 base pairs in exons 1, 4, and 11. One of the mutations was a single base substitution creating a stop codon in exon 10. The fifth mutation was a missense mutation in exon 8. This variant has been reported previously.44 Our series therefore confirms that mutations in DPC4 are associated with JPS but the mutations we detected did not involve the trimerisation domain of the DPC4 protein. There were no distinguishing clinicopathological features in those patients harbouring DPC4 mutations.

Discussion

Several syndromes—JPS, CS, BRRS, GS, and HMPS—include juvenile polyps in their phenotype. These diseases can have subtle manifestations and some of their clinical features are not unique. Hence diagnosis is not straightforward. However, it is important to distinguish between these syndromes as the types of cancer associated with each appear to be quite different. A more reliable and objective means of differentiating syndromes than reliance solely on clinical features is clearly desirable, especially in the case of JPS the diagnosis of which is made in part by exclusion. Determining the molecular basis of each of these syndromes clearly offers the best method of establishing a diagnosis.

Until recently the molecular basis of the hamartomatosis polyposis syndromes was unknown. Early somatic data suggested the existence of a putative locus for JPS at 10q22–24, encompassing PTEN.17 This observation led several researchers to examine PTEN as a candidate for JPS. No linkage to 10q22 or mutation in PTEN was detected in either the JPS families or isolated cases studied by Marsh and colleagues.20 However, both Olschwang and colleagues21 and Lynch and colleagues30 reported germline PTEN mutations in patients with multiple juvenile polyps, suggesting that PTEN was the cause of some JPS cases. Eng has recently questioned their conclusion.22 In the study by Olschwang and colleagues,21 the adult male patient (G116) also had a laryngeal carcinoma and a heterogeneous thyroid nodule, which is highly suggestive of CS. Furthermore, although both children in the report (G796 and G7710) did not have features indicative of CS or BRRS, they were less than 15 years of age. As the penetrance of CS is only 10% by this age23 it is possible that both children will develop features of CS later in life. Similarly, the affected members of the family reported by Lynch and colleagues30 had major clinical features consistent with a diagnosis of CS.

Our mutational analysis of JPS patients is the most comprehensive to date. The findings concur with previous work suggesting that the probability of detecting a constitutive mutation in PTEN is not high if a patient’s clinical phenotype does not adhere to the International Cowden Consortium operational diagnostic criteria for CS30 or to the specific clinical features of BRRS.

PTCH is a good candidate for JPS but has not previously been tested for germline mutations in JPS patients. We have excluded this gene as a cause of juvenile polyps outside the setting of GS. Although PTCH mutations may directly lead to gastrointestinal hamartoma formation it remains possible that reports of juvenile polyps in GS result from a chance association or from contiguous deletion of PTCH and at least one other gene.

Recent work has identified germline mutations in the DPC4 (SMAD4) gene on 18q21.1 as a cause of some cases of JPS.33 In our study 21% of JPS could be ascribed to germline mutations in DPC4 (five of 24; comprising 15 families and nine sporadic cases tested). This provides further support for the role of mutations in this gene as a cause of JPS. Failure to detect mutations in DPC4 in the other patients is unlikely to reflect problems in the screening method alone: it is possible that some mutations may be in the UTRs, introns, or promoter region of the gene but we have screened all exons and splice sites and have found that CSGE can detect all small insertions, deletions, and 90% of single base substitutions under such conditions.

DPC4/SMAD4 is a pivotal component of the transforming growth factor β signal transduction pathway.4 Through hetero-oligomer formation by interaction with SMAD1 (and possibly 5) and SMAD2 (and possibly 3), SMAD4 mediates apoptotic and growth inhibition responses. Hamartoma formation in JPS presumably results from disruption of the transforming growth factor β signal transduction pathway.35 In our study mutations in SMAD4 were detected in exons 1, 4, 8, 10, and 11. Pathogenic mutations previously reported were in exons 5, 8, and 9.35 A wide range of mutations can clearly affect cellular responses to transforming growth factor β leading to hamartoma formation. Whether specific genotype-phenotype relationships exist await further studies.
We conclude that the clinical features of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Gorlin syndrome can be used to distinguish patients who are likely to carry germline mutations in *PTEN* and *PTCH* and who do not, therefore, have a diagnosis of JPS. The rare syndrome HMPS can probably be distinguished from JPS on histological grounds, and there is no evidence for linkage of JPS to the HMPS locus (although this cannot be confirmed until the HMPS gene is identified). Combining our findings and those reported by Howe and colleagues suggests that up to 40% of JPS cases might be caused by germline mutations in *DPC4* although these patients cannot be distinguished from the majority of JPS patients who harbour germline mutations in uncharacterised genes. Current evidence does not suggest any common mode of action of *PTEN*, *PTCH*, and *DPC4*. Therefore, there are as few clues as to the nature of the uncharacterised genes for JPS as it appears that several different cellular defects are associated with hamartomatous juvenile polyps of identical appearance.

We thank the patients that took part in this study. Part of this work was supported by BREAKTHROUGH Breast Cancer. KW-R is supported by the EEC, SB is in receipt of a Fellowship from the Coeliac Society, MC is supported by the CRC, and BD by the MRC.

Analysis of genetic and phenotypic heterogeneity in juvenile polyposis


Gut 2000 46: 656-660
doi: 10.1136/gut.46.5.656

Updated information and services can be found at:
http://gut.bmj.com/content/46/5/656

These include:

References
This article cites 33 articles, 14 of which you can access for free at:
http://gut.bmj.com/content/46/5/656#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/